REMARKS

Claims 1, 2, 4-8 and 12-16 are currently pending. A list of the currently pending claims is provided in an appendix for the Examiner's convenience.

A request has been made for the amendments submitted March 13, 2001 to be entered. In the Final Office Action mailed November 13, 2000, Claims 1, 2, 4-7 and 15 were rejected under 35 U.S.C. § 112, second paragraph for reciting a phrase that lacked antecedent basis. Applicants believe that the amendments mentioned above and arguments submitted therewith adequately addressed this rejection. This is supported by the Advisory Action mailed April 3, 2001, since these amendments and accompanying arguments are not cited as having been considered but NOT placing the application in condition for allowance.

Claims 8, 13, 14 and 16 were rejected under 35 U.S.C. § 102(b) as being anticipated by Stemple et al., *Cell* 71:973-985 (1992) (Stemple I), which rejection Applicants traversed in the response submitted March 13, 2001. Applicants believe that the arguments submitted therein adequately addressed this rejection. As with the definiteness rejection, this belief is supported by the Advisory Action mailed April 3, 2001, since these amendments and accompanying arguments are not cited as having been considered but NOT placing the application in condition for allowance.

Rejection Under 35 U.S.C. § 103

Claims 1, 2, 4-8 and 12-16 are rejected under 35 U.S.C. § 103(a) as being obvious over Lo et al., *Perspectives Dev. Neurobiol.* 2:191-201 (1994) (Lo), Stemple *et al.*, *Dev. Biol.* 159:12-23 (1993) (Stemple II), Stemple *et al.*, *Cell* 71:973-985 (1992.) (Stemple I), and Martucciello *et al.*, *J. Ped. Surg.* 30(3):433-436 (1995) (Martucciello). Applicants respectfully traverse.

For a rejection under 35 U.S.C. § 103 to be proper, it must be shown that: 1) each element of a claim is disclosed or suggested in the prior art; 2) the prior art provided motivation to combine and/or modify prior art disclosures to obtain the claimed invention; and 3) the skilled artisan would have a reasonable expectation of successfully obtaining the claimed invention. Applicants submit that all of these have not been met in the present rejection.

Claims 1 and 2 are directed to a composition comprising a monoclonal antibody and a cell selected from the group consisting of three specific cell types: a multipotent neuronal progenitor (proNP) cell, a nonneuronal progenitor (NNP) cell and a committed neuronal progenitor (NP) cell, each of which comprise RET protein. The antibody is specifically bound to at least a portion of the RET protein. Claims 4-7 and 15 are directed to a method for enrichment of neural progenitor cells comprising RET protein. The method comprises combining neural crest derived cells comprising neural progenitor cells with an antibody and isolating RET positive cells. Claims 8, 12-14 and 16 are directed to a substantially pure population of neural crest derived neural progenitor cells prepared using antibody binding to RET protein. Applicants submit that none of the references, alone or in any combination, teach that RET protein is expressed in any of the described cell types, nor that such cells could be bound to antibodies to RET protein or enriched using such antibodies.

Lo discloses labeling of cells by *in situ* hybridization of cRNA probes for *MASH-1* and *c-ret* mRNA in developing sympathetic and enteric ganglia and adrenal gland. This reference provides no evidence of expression of the RET protein. The Office Action mailed 4/1/98 (the only Office Action making any specific reference to the cited art itself), states that Lo teaches RET as a valuable marker for early stages of neural crest cell lineage diversification. Applicants point out that Lo refer only to the *c-ret* gene as a marker, not the protein, and provide no suggestion as to how cells expressing this gene might be isolated. In the Advisory Action mailed April 3, 2001, the Examiner insists that "one would have expected protein present from expression of encoding mRNA." However, even if, arguendo, such an expectation exists, Lo still provides the skilled artisan with no indication that the protein encoded by the labeled mRNA would be expressed at the surface of the cells, which would be required for binding of the antibodies of the claims with the specified cells.

And even if, arguendo, the skilled artisan would expect the RET protein to be expressed at the cell surface, Lo provides no indication that sufficient numbers of the protein would be expressed so that such cells could be identified and selected on the basis of antibody binding. The mRNA that was labeled by Lo was not isolated and transcribed to confirm that it encoded the RET protein. No information is provided as to whether or how much of the protein is

produced in the cells. Applicants submit that this reference fails to show RET protein positive cells of any kind.

Furthermore, even if, arguendo, the disclosure of Lo were interpreted to show cells which express the RET protein, neither this reference nor any of the other references, alone or in any combination, disclose beyond pure speculation any of the cell types specified in the claims as expressing the RET protein. Lo discloses cells labeled by *in situ* hybridization. But, while the labeled cells are described as being in the proper location to be involved in the make up of certain ganglia or glands, no definitive conclusion can be drawn as to what types of cells are actually labeled. The areas in which the labeled cells were located would comprise a multitude of different cell types. Lo states that it is not known whether the same cells that express MASH-1 (an autonomic neuronal cell marker) also express *c-ret*, but postulate that they are from the same population of cells (page 194, first full paragraph). This could mean that *c-ret* cells are neuronal progenitors or neuronal cells only. The authors also admit that the labeled cells could be from two distinct but comingled populations. This could mean that *c-ret* cells are non-neuronal precursors only. What Lo teaches are possibilities which would require significant further investigation to determine which of the possibilities are reality. What is presented in Lo regarding the cells identified by *c-ret* labeling is, by their own admission, purely speculative. This reference can only be construed as suggesting that the *c-ret* gene marker might be a useful tool for investigating cell lineage, with no actual disclosure of what types of cells would actually be labeled if such investigation were undertaken. Thus, Lo does not and cannot disclose the compositions, cell populations and methods that are claimed.

Stemple II does not cure the deficiencies of Lo. This reference provides a review of the influences on developing cells and references the various approaches that have been taken in such study. While this reference does describe uses of antibodies in this research, Stemple II does not mention RET protein, let alone what types of cells might express the protein. Furthermore, Stemple II provides a caveat for those using an antibody approach, noting that "antigenic heterogeneity in neural crest cell populations cannot necessarily be taken as evidence of heterogeneity in developmental potential." (Page 17, right column, first full paragraph). Applying this caveat to the findings of Lo, that some cells express MASH-1 RNA and some

express *c-ret* does not provide definitive information as to what cell types they label or their potential. One cannot tell whether the *c-ret*+ cells are destined to be neuronal, non-neuronal or both, merely because they co-locate with MAST-1+ cells.

Stemple I discloses isolating neural crest cells with an antibody to the low affinity nerve growth factor (LNGFR). This reference does not disclose RET protein, nor does it possibly indicate what cell types would be labeled with an antibody to the RET protein. While Stemple I provides general direction regarding an approach that may be taken to finally obtain the information necessary to conceive the presently claimed invention, the invention itself cannot be derived from this reference, alone or in combination with any of the other cited references.

Martucciello describes antibody labeling of RET protein in diseased and normal adult human tissue . This reference describes RET as a protein expressed in human tumors. This reference provides no evidence of the mode or even the fact of expression of this protein in developing cells, let alone what types of cells would be labeled with the antibody.

All Elements Not Taught

Each of the composition, cell population and methods claims list specific cell types expressing RET protein. None of the cited references show what specific cell types express RET, let alone specify that the cell types of the present claims can be labeled and isolated using an antibody to RET. Without the inventive effort performed by the present inventors, this information was not and could not have been known.

Furthermore, in all of the present claims, there is the element of a neural progenitor cell comprising RET protein such that an antibody specific thereto can bind the protein. None of the cited references, alone or in any combination, teach neural crest cells expressing RET protein. Nor do any of these references teach expression of RET protein in neural crest cells such that it may be bound by an antibody. Prior to the present disclosure, it was not known that developing neural crest cells expressed the RET protein, nor that the RET protein was expressed at the cell surface, nor that the RET protein presented an antigen that could be bound by an antibody on the

cell surface and used to perform the methods and provide the compositions and cell populations of the present claims.

In addition, none of the cited references teach that neural progenitor cells, a distinct and specific subset of neural crest derived cells, express RET protein or express the protein such that an antibody can bind at the cell surface so that it may be used to enrich a population of cells with neural progenitor cells or to prepare a substantially pure population of neural progenitor cells.

The lack of disclosure of these claim elements has been brought up before in the prosecution history of this application and has not been addressed in subsequent Office Actions. The criteria to support a *prima facie* case for obviousness are specific and all of these criteria must be met. Repeated insistence that the skilled artisan would be motivated to combine references does not make up for the shortcomings of the references with regard to the other two criteria.

No Motivation to Combine or Modify Prior Art to Provide the Present Invention

The Office Action mailed April 1, 1998 suggests that it would be obvious to use antibodies such as disclosed in Martucciello for immunological fractionation of RET+ cells by conventional methods such as disclosed in Stemple I because Stemple II says such methods have been useful and Lo teaches that RET is a useful marker for early neural crest cell lineage (page 12 of Office Action mailed 4/1/98). However, this combination of references does not provide the present invention. All a combination of these references provides are methods for obtaining the information necessary to conceive the present invention. Without the knowledge that RET protein is in fact expressed in developing neural crest cells, that antibodies could be obtained to label neural cells, and that cells so labeled are multipotent neuronal progenitor cells, committed neuronal progenitor cells and nonneuronal progenitor cells, the skilled artisan cannot conceive of the present invention.

The Office Action analysis takes a giant leap past the absence of disclosure that developing neural crest cells express RET protein or that such RET protein, if expressed, could be labeled by antibodies. Furthermore, the Office Action takes a second leap to suggest that such labeling would obviously allow for the preparation of a composition or population of cells

comprising neural progenitor cells. The combination of references cited provides for the investigation of the lineage of RET protein-expressing cells in tumors and Hirschsprung's disease, not the present invention.

No Reasonable Expectation of Success

As discussed above, the lack of information regarding RET in neural crest derived neural progenitor cells left inherent uncertainties at the time of invention as to whether these cells expressed the RET protein or whether, even if they did, that it would be sufficiently antigenic to allow antibodies to specifically bind so as to permit enrichment of such cells. Furthermore, the lack of evidence as to what types of cells may actually express RET protein at all, regardless of whether it would be useful for isolation of cells, leaves the skilled artisan incapable of expecting to obtain the claimed invention. With this inherent uncertainty, the ordinary skilled artisan would not have a reasonable expectation that the claimed methods for enrichment, or compositions or populations of cells would result, even if the teachings of the cited art were combined and used. This last fact is further supported by the statements in Stemple II, warning that immunogenic heterogeneity does not necessarily suggest heterogeneity in developmental potential. The neural progenitor cells of the present claims have a distinct developmental potential that could not be predicted to be different from, for example, LNGFR expressing cells, even if it was known that neural crest cells did express RET protein in the manner necessary to obtain the present invention.

The Examiner repeatedly asserts that the references provide motivation to isolate RET+ cells for further research (e.g., Office Action mailed 11/13/00, pages 5-6; Office Action mailed 2/28/00, pages 4-5; Office Action mailed 10/26/98, pages 9-10), however, the claims are not directed to methods of isolating RET+ cells. Rather, the claims are directed to specific compositions, cell populations and methods for enrichment of specific cell types which could only be known from the further inventive effort of investigating the expression of the RET protein, determination of the cell types labeled thereby, and extrapolating the utility of such information. The Examiner does not address the claimed invention, only the process by which one might obtain the information that would be necessary to conceive the invention.

For the reasons discussed above, Claims 1, 2, 4-8, and 12-16 are not obvious over Lo, Stemple II, Stemple I and Martuciello. Therefore, Applicants respectfully request that this 35 U.S.C. § 103(a) rejection be withdrawn.

CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and an early notification of such is solicited. If the Examiner believes a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned attorney.

Respectfully submitted,

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APPENDIX

1. (Four Times Amended) A composition comprising a monoclonal antibody and a cell selected from the group consisting of a multipotent neuronal progenitor (proNP) cell, a nonneuronal progenitor (NNP) cell and a committed neuronal progenitor (NP) cell, each of which comprise RET protein, wherein said monoclonal antibody is specifically bound to all or part of a sequence of said RET protein on said cell.

2. (Twice Amended) The composition according to claim 1, wherein said sequence consists essentially of the extracellular domain of RET.

4. (Four Times Amended) A method for the enrichment of neural progenitor cells comprising RET protein, said method comprising:

a) combining a mixed population of cells comprising neural-crest derived cells comprising neural progenitor cells with an antibody that specifically binds to all of part of a sequence of said RET protein; and

b) selecting for RET positive cells, whereby the percentage of neural progenitor cells is enriched.

5. (Amended) The method according to claim 4 wherein said antibody is selected from the group consisting of polyclonal antibody, monoclonal antibody, antibody fragments, and single chain antibody.

6. (Amended) The method according to claim 5, wherein said antibody is fluorochrome conjugated.

7. (Twice Amended) A method according to claim 6, wherein said selecting with said fluorochrome conjugated antibody is by flow cytometry.

8. (Four Times Amended) The population according to Claim 16, wherein said cells are nonneuronal progenitor (NNP) cells.

12. (Amended) The population according to claim 16 wherein said neural progenitor cells are bound to an antibody that specifically binds to RET antigen.

13. (Twice Amended) The population according to claim 12 or 16 wherein said antibody is selected from the group consisting of polyclonal antibody, monoclonal antibody, antibody fragments, and single chain antibody.

14. (Amended) The population according to claim 13 wherein said antibody is a monoclonal antibody.

15. (Thrice Amended) A method for the enrichment of neural progenitor cells, said method comprising:

a) combining a mixed population of cells comprising neural-crest derived cells comprising neural progenitor cells comprising RET protein with a monoclonal antibody that specifically binds to all of part of a sequence of said RET protein; and

b) selecting for RET positive cells, whereby the percentage of neural progenitor cells is enriched.

16. (Twice Amended) A substantially pure population of neural crest derived neural progenitor cells comprising RET protein prepared using antibody binding to RET protein, where said cells are proneuronal progenitor (proNP) cells, neuronal progenitor (NP) cells and/or nonneuronal progenitor (NNP) cells.